

tor™ and in the stirred tank bioreactor were comparable at $\sim 0.5 \text{ day}^{-1}$. Despite the lack of online feedback control for pH and DO in the WAVE BIOREACTOR™ system, the pH and DO profiles did not differ significantly between the two bioreactor cultures. The invention provides a process control method for maintaining culture pH in the 6.8-7.2 range, and DO >20% of air saturation in the WAVE BIOREACTOR™ system—operated in both batch and perfusion modes—without relying on pH and DO feedback control. After identifying challenges in culturing CHO cells in the WAVE BIOREACTOR™ system without pH and DO control, we conducted cell-free studies to determine the effects of rock rate, rock angle, and gas flow rate on O_2 and CO_2 transfer in the WAVE BIOREACTOR™ system. By adjusting these process parameters along with the concentration of CO_2 and O_2 in the inlet gas, we maintained culture pH and DO within our desired range for batch and perfusion cultures of six recombinant CHO cell lines. By eliminating the need for pH and DO probes, this process provides a simpler and more cost-effective method for culturing cells in the WAVE BIOREACTOR™ system. It also provides an alternative method for culturing cells in the event of pH or DO probe failure in WAVE BIOREACTORS™ equipped with these probes.

[0145] It is also to be understood that the specific examples described herein are illustrative only and not intended to limit the scope of the invention. The invention is limited only by the appended claims.

REFERENCES CITED

- [0146] Cronin C N, Lim K B, Rogers J. 2007. Production of selenomethionyl-derivatized proteins in baculovirus-infected insect cells. *Protein Sci* 16: 2023-2029.
- [0147] Dunn I J, Einsele A J. 1975. Oxygen transfer coefficients by the dynamic model. *J Appl Chem Biotechnol* 25: 707-720.
- [0148] deZengotita V M, Schmelzer A E, Miller W M. 2002. Characterization of hybridoma cell responses to elevated pCO_2 and osmolality: Intracellular pH, cell size, apoptosis, and metabolism. *Biotechnol Bioeng* 77: 369-380.
- [0149] Haldankar R, Li D, Saremi Z, Baikalov C, Deshpande R. 2006. Serum-free suspension large-scale transient transfection of CHO cells in WAVE bioreactors. *Mol Biotechnol* 34: 191-199.
- [0150] Johnson M, Lanthier S, Massie B, Lefebvre G and Kamen A. 1996. Use of the Centritech lab centrifuge for perfusion culture of hybridoma cells in protein-free medium. *Biotech Prog* 12: 855-864.
- [0151] Langheinrich C and Nienow A W. 1999. Control of pH in large-scale, free suspension animal cell bioreactors: Alkali addition and pH excursions. *Biotechnol Bioeng* 66: 171-179.
- [0152] Lin A, Kimura R, Miller W M. 1993. Production of tPA in rem binant CHO cells under oxygen-limited conditions. *Biotechnol Bioeng* 42: 339-350.
- [0153] Ling W L W, Deng L, Lepore J, Cutler C, Connon-Carlson S, Wang Y, Voloch M. 2003. Improvement of monoclonal antibody production in hybridoma cells by dimethyl sulfoxide. *Biotechnol Prog* 19: 15-162.
- [0154] Link T, Bäckström M, Graham R, Essers R, Zörner K, Gätgens J, Burchell J, Taylor-Papadimitriou J, Hansson G C, Noll T. 2004. Bioprocess development for the production of a recombinant MUC1 fusion protein expressed by CHO-K1 cells in protein-free medium. *J Biotechnol* 110: 51-62.
- [0155] Miller W M, Blanch H W, Wilke C R. 1988. A kinetic analysis of hybridoma growth and metabolism in batch and continuous suspension culture: Effect of nutrient concentration, dilution rate, and pH. *Biotechnol Bioeng* 32: 947-965.
- [0156] Mikola M, Seto J, Amanullah A. 2007. Evaluation of a novel Wave Bioreactor cell bag for aerobic yeast cultivation. *Bioprocess Biosyst Eng* 30: 231-241.
- [0157] Osman J J, Birch J, Varley J. 2001. The response of GS-NSO myeloma cells to pH shifts and pH perturbations. *Biotechnol Bioeng* 75: 63-73.
- [0158] Osman J. I. Birch .1, Varley J. 2002. The response of GS-NSO myeloma cells to single and multiple pH perturbations. *Biotechnol Bioeng* 79: 39-407.
- [0159] Rao G, Moreira A, Brorson K. 2009. Disposable bioprocessing: the future has arrived. *Biotechnol Bioeng* 102: 348-356.
- [0160] Restelli V, Wang M D, Huzel N, Ethier M, Perreault H, Butler M. 2006. The effect of dissolved oxygen on the production and glycosylation profile of recombinant human erythropoietin produced from CHO cells. *Biotechnol Bioeng* 94: 481-494.
- [0161] Royce P N C, Thornhill N F. 1991. Estimation of dissolved carbon dioxide concentrations in aerobic fermentations. *AIChE* 37L 1680-1686.
- [0162] Singh V. 1999. Disposable bioreactor for cell culture using wave-induced agitation. *Cytotechnology* 30: 149-158.
- [0163] Tang Y J, Ohashi R, Hamel J F P. 2007. Perfusion culture of hybridoma cells for hyperproduction of IgG_{2a} monoclonal antibody in a Wave bioreactor-perfusion culture system. *Biotechnol Bioeng* 23: 255-264.
1. A method for batch culturing eukaryote cells comprising:
- providing cell culture inoculant comprising eukaryotic cells in a bicarbonate-containing culture liquid to a vessel,
 - said vessel having walls that encapsulate said cell culture and a gas phase head space above said cell culture, and wherein said vessel comprises at least one port that provides an entrance and an egress of gas to and from said head space;
 - agitating said vessel; and
 - providing gas to said head space through said port wherein said gas contains an amount of CO_2 , and wherein said amount of CO_2 in said gas is modulated over time to adjust the pH of said cell culture in order to maintain a predetermined pH of said cell culture.
- 2-11. (canceled)
12. A method for perfusion culturing eukaryote cells comprising:
- providing cell culture inoculant comprising eukaryotic cells in a bicarbonate-containing culture liquid to a vessel,
 - said vessel having walls that encapsulate said cell culture and a gas phase head space above said cell culture, and wherein said vessel comprises at least one port that provides an entrance and an egress of gas to and from said head space;
 - agitating said vessel;